Food Bioscience 56 (2023) 103074

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Antioxidants location affects the oxidative stability of spray-dried microcapsules loaded with fish oil

Nor E. Rahmani-Manglano *, Pedro J. García-Moreno **, Raúl Pérez-Gálvez, Emilia M. Guadix

Department of Chemical Engineering, University of Granada, 18071, Granada, Spain

ARTICLE INFO

Keywords:
Omega-3 polyunsaturated fatty acids
Lipid oxidation
Natural antioxidants
Protein hydrolysate
Spray-drying
Encapsulation

ABSTRACT

The oxidative stability of fish oil-loaded capsules (~15 wt% oil load) produced by spray-drying and containing natural antioxidants of different polarity was investigated. For this purpose, three commercial rosemary extracts (e.g., water-dispersible; K1 or oil-soluble; K2, K3) and whey protein concentrate hydrolysate (WPCH), exhibiting surface-active properties, were evaluated. The capsules showed similar physicochemical properties (e.g., morphology, size and encapsulation efficiency >91%), regardless of the formulation. However, the polarity of the antioxidant used significantly influenced the oxidative stability. The microcapsules containing hydrophobic antioxidants showed the lowest peroxide value (PV) after drying, followed by the WPCH-containing capsules, although the PV evolution over storage was similar among the samples. Nonetheless, WPCH was the most effective antioxidant reducing the formation of volatile secondary oxidation products in the capsules. This was attributed to its partition at the oil/matrix interface, resulting in an enhanced protective effect when combined with tocopherols present in the encapsulated oil.

1. Introduction

Food fortification with omega-3 polyunsaturated fatty acids (PUFAs) has gained great interest over the last decades due to the health benefits attributed to their intake, especially to EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3) (Patel et al., 2022; Sandhya, Leena, Moses, & Anandharamakrishnan, 2023). However, their inclusion into complex food matrices is still challenging for the food industry, mainly because of their low oxidative stability. As it has been extensively reported, lipid oxidation of omega-3 PUFAs results in the loss of their nutritional properties and also has an impact on their organoleptic properties (e.g., development of flavor/odor active compounds) (Arab-Tehrany et al., 2012; Baik et al., 2004; Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016; Velasco, Dobarganes, & Márquez-Ruiz, 2003), both lowering the quality of the enriched food product. Therefore, the addition of omega-3 rich oils (e.g., fish oil) in the form of a delivery system has been proposed as a promising approach to enhance the oxidative stability of both, the omega-3 PUFAs and the fortified foodstuff (Sørensen, García-Moreno, Yesiltas, & Jacobsen, 2021). In this regard, the use of encapsulation technologies for the development of efficient dried omega-3 delivery systems (e.g., fish oil-loaded capsules) is of special interest.

Spray-drying is the most common encapsulation technique used for the food industry and it has been extensively used in the production of fish oil-loaded encapsulates aimed as omega-3 delivery systems (Rahmani-Manglano et al., 2020). Encapsulation by spray-drying entraps the fish oil within a glassy encapsulating matrix, generally carbohydrate-based, that acts as a physical barrier against environmental prooxidants (e.g., oxygen) aimed to prevent its degradation. However, it has been demonstrated that the onset of lipid oxidation already occurs during the encapsulation process (Baik et al., 2004; Serfert, Drusch, & Schwarz, 2009). The intense mechanical stress exerted during the emulsification step (i.e., production of a coarse emulsion followed by a homogenization step) leads to the inclusion and distribution of prooxidant species (e.g., oxygen, metal traces) within the system and, in most cases, this process is accompanied by a significant temperature increase (Berton-Carabin, Ropers, & Genot, 2014). Furthermore, since drying is carried out at high temperatures (inlet/outlet temperature ranging from 200 to 80 °C, respectively), lipid oxidation is also likely to occur during spray-drying if the process is not efficiently designed (e.g., low encapsulation efficiency and high residence time of the capsules in the drying chamber) (Santos et al., 2018).

* Corresponding author.
** Corresponding author.
E-mail addresses: norelenarm@ugr.es (N.E. Rahmani-Manglano), pjgarcia@ugr.es (P.J. García-Moreno).

https://doi.org/10.1016/j.fbio.2023.103074
Received 12 June 2023; Received in revised form 1 August 2023; Accepted 24 August 2023
Available online 25 August 2023

The initial oxidative status of the encapsulated oils (right after drying) is of outmost importance as it will further influence the oxidative stability of the encapsulated system during storage since, once lipid radicals are present in the oil, lipid oxidation rapidly propagates (Johnson & Decker, 2015). A strategy to minimize lipid oxidation during processing, and in consequence enhance the oxidative stability of the capsules during storage, is the addition of antioxidants to the formulation (Serfert et al., 2009). Nowadays, the focus is placed on finding natural ingredients with antioxidant activity (e.g., plant extracts or protein hydrolysates) since, not only they are better considered by the consumer in terms of clean label and sustainability, but also allows to avoid the health hazards related to traditional synthetic antioxidants (e.g., butylated hydroxylanisole (BHA) or butylated hydroxytoluene (BHT), among others) (Hrebień-Filisińska, 2021). Natural plant-derived antioxidants, such as rosemary extract, are the most frequently used in the stabilization of fish oil (Anwar & Qadir, 2021; Hrebień-Filisińska, 2021). The antioxidant activity of rosemary extract is related to its content of carnosic acid (CA) and its oxidation product, carnosol (CN), which act as efficient radical scavengers (Hopia, Huang, Schwarz, German, & Frankel, 1996). Furthermore, CA has been reported to exhibit protective effect towards tocopherols (e.g., α-tocopherol or δ-tocopherol) during lipid oxidation, thus acting as a synergistic antioxidant (García-Moreno, Damberg, Chronakis, & Jacobsen, 2017; Hopia et al., 1996). Additionally, protein hydrolysates have shown high potential as antioxidants, gaining more attention in the last years the use of peptides as natural antioxidants in food (Hrebień-Filisińska, 2021).

Several studies carried out in wet heterogeneous systems (i.e., oil-in-water emulsions) have shown that one of the most important factors influencing the efficacy of an antioxidant is its partition in the system, which is influenced by its polarity (e.g., lipophilic, amphiphilic or hydrophilic) (Laguerre et al., 2015; Shahidi & Zhong, 2011). Generally, it is assumed that antioxidants with a tendency to accumulate at the oil/water interface (e.g., amphiphilic antioxidants) are more efficient in wet emulsified systems since this microenvironment has been proposed to be the place where lipid oxidation is initiated (i.e., contact area between prooxidants and the oil) (Aslam & Schroën, 2023; Berton-Carabin et al., 2014; Laguerre et al., 2015; Shahidi & Zhong, 2011). Therefore, although the behaviour of antioxidants in dried heterogeneous systems is not fully understood yet (Baik et al., 2004; ten Klooster et al., 2022; Velasco et al., 2003), it is plausible to speculate that for dried emulsions, such as oil-loaded capsules, the antioxidant activity might also be strongly influenced by its location within the dehydrated system (e.g., at the encapsulated oil droplets, at the oil/matrix interface or at the glassy matrix) (Velasco et al., 2003). In this line, a recent study evaluated the influence of the polarity of gallic acid esters on the oxidative stability of spray-dried oil-loaded capsules (ten Klooster et al., 2022). Interestingly, propyl gallate exhibiting amphiphilic properties did not significantly enhanced the oxidative stability of the encapsulated oil contrary to what could be expected based on its antioxidant activity in wet oil-in-water emulsions (ten Klooster et al., 2022). On the contrary, these authors reported that the more non-polar gallates (i.e., octyl, lauryl and hexadecyl gallate) were more efficient preventing lipid oxidation of the encapsulated oil, being the latter attributed to their partition within the oil phase of the capsules. It should be borne in mind, however, that the antioxidant activity of gallic acid and its derivatives arise from the radical scavenger activity of the polar phenolic head (Garrido, Garrido, & Borges, 2012). Unfortunately, the effect of amphiphilic compounds with antioxidant activity in both their polar and non-polar part (i.e., peptides) on the oxidative stability of dry heterogeneous systems has not been investigated to date.

Therefore, taken altogether, the aim of the study was to investigate the oxidative stability of fish oil-loaded capsules produced by spray-drying containing natural antioxidants of different polarity. For this purpose, the capsules were produced using three different commercial antioxidants: i) a water-dispersible rosemary extract, ii) an oil-soluble rosemary extract, iii) an oil-soluble mixture of antioxidants including rosemary extract, tocopherols, ascorbic acid, citric acid and lecithin. Moreover, whey protein concentrate hydrolysate (WPCH, degree hydrolysis of 10%), which was previously reported to exhibit high antioxidant and emulsifying activity (Padial-Domínguez, Espejo-Carpio, García-Moreno, Jacobsen, & Guadix, 2020; Padial-Domínguez, Espejo-Carpio, Pérez-Gálvez, Guadix, & Guadix, 2020), was also evaluated as amphiphilic antioxidant. The results of this work will provide new insights on the effect of antioxidants location within dried emulsions and their ability to retard lipid oxidation.

2. Materials and methods

2.1. Materials

Omega Oil 1812 TG Gold with a peroxide value (PV) of 0.88 ± 0.02 meq O_2/kg oil (measured as described in Section 2.4.1), was purchased from BASF Personal Care and Nutrition GmbH (Illertissen, Germany) and stored at −80 °C until use. The tocopherol content was determined as described in our previous work (Rahmani-Manglano et al., 2020) and was as follows: alpha-, gamma- and delta-tocopherol content of 500.8 ± 1.3, 2108.8 ± 123 5.3 and 677.4 ± 2.0 μg/g oil, respectively. Glucose syrup (GS; DE38, C*Dry 1934) was kindly donated by Cargill Germany GmbH (Krefeld, Germany). Tween 20 (T20) was purchased from Sigma-Aldrich (Darmstadt, Germany). The three rosemary-based extracts of different polarities under the commercial name of: Herbalox® HT-P (K1, water-dispersible), Herbalox® XT-O (K2, lipid-soluble) and Duralox® MAN-5 (K3, lipid-soluble) were kindly donated by Kalsec, Inc. The whey protein concentrate hydrolysate (WPCH), also used as an antioxidant, was produced by enzymatic hydrolysis with Alcalase® 2.4L (Novozymes, Bagsvaerd, Denmark) to a degree of hydrolysis (DH) of 10%, as described elsewhere (Rahmani-Manglano et al., 2020). The hydrolysis conditions (i.e., enzyme used, pH and DH) were set based on previous studies carried out by our research group so that the emulsifying and the antioxidant activity were maximized (Padial-Domínguez, Espejo-Carpio, García-Moreno, et al., 2020; Padial-Domínguez, Espejo-Carpio, Pérez-Gálvez, et al., 2020). The rest of the reagents used were of analytical grade.

2.2. Production of the capsules

Fish oil-in-water emulsions containing one of the aforementioned antioxidants (K1, K2, K3 or WPCH) were subjected to spray-drying to produce the capsules (~15 wt% oil load), as reported in our previous work (Rahmani-Manglano et al., 2020). The antioxidant concentration in the emulsions was fixed to 200 ppm of carnosic acid and carnosol (CA + CN) for the rosemary extract-based antioxidants (K1, K2, K3) or 200 ppm of protein in case of the protein-based antioxidant, WPCH, with respect to fish oil. A control sample without antioxidants was also produced. The aqueous phase of the emulsions was produced by dissolving the encapsulating agent (GS, 28 wt%) and the emulsifier (T20, 0.35 wt %) in distilled water, and stirring overnight at ambient temperature. Then, a coarse emulsion was produced by dispersing the fish oil (5 wt%) in the aqueous phase during the first minute of mixing at 15,000 rpm using an Ultraturrax T-25 homogenizer (IKA, Staufen, Germany). The total processing time was 2 min. The polar antioxidants (K1 and WPCH) were added to the aqueous phase, whereas the non-polar antioxidants (K2 and K3) where added to the oil. The coarse emulsion was further processed in a high-pressure homogenizer at a pressure range of 450/75 bar, applying 3 passes (PandaPLUS 2000; GEA Niro Soavi, Lübeck, Germany). Spray-drying was conducted in a pilot plant scale spray drier (Mobile Minor; Niro A/S, Copenhagen, Denmark) at 190/80 °C inlet/outlet temperature, respectively, with the pressure of the pneumatic air activating the rotary atomizer set to 4 bar (22,000 rpm). The drying air flow was ~60 Nm3/h and the infeed emulsion flow rate was fixed to 1.5 kg/h.

2.3. Characterization of the spray-dried microcapsules

2.3.1. Oil droplet size distribution (ODSD)

The oil droplet size distribution (ODSD) of the emulsions before (parent emulsions) and after drying (reconstituted emulsions) was measured by laser diffraction in a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK) as previously reported by Rahmani-Manglano, Tirado-Delgado, García-Moreno, Guadix, and Guadix (2022). The emulsions were reconstituted by dissolving the microcapsules in distilled water in order to obtain the same solid content as the original emulsion. Then, the samples were diluted in recirculating water (3000 rpm) to achieve an obscuration in the range 12–15%. The refractive indexes of fish oil (1.481) and water (1.330) were used as particle and dispersant, respectively. Measurements were made in triplicate.

2.3.2. Morphology and size

A thin layer of powder was placed on carbon tape and carbon-coated (EMITECH K975X Turbo-Pumped Thermal Evaporator, Quorum Technologies, UK) to investigate their morphology and size by scanning electron microscopy (SEM) using a FESEM microscope (LEO 1500 GEMINI, Zeiss, Germany) (Rahmani-Manglano, Tirado-Delgado, et al., 2022). The SEM images were acquired in the range 250X – 2KX magnification with a 5-kV accelerating voltage. The ImageJ software (National Institute of Health) was used to analyze the images. Approximately, 135 randomly-selected capsules were measured to determine the particle size distributions and mean diameters.

2.3.3. Encapsulation efficiency (EE)

The encapsulation efficiency (EE) was determined by extracting the non-encapsulated surface oil as described elsewhere (Rahmani-Manglano, Tirado-Delgado, et al., 2022). Briefly, 2.5 g of powder was weighed and mixed with 15 mL of hexane in a vortex mixer for 2 min and then centrifuged at 2720g for 20 min. Then, 5 mL of supernatant were collected in a Pyrex tube (previously weighted) and evaporated under a constant flow of nitrogen. Afterwards, the Pyrex tube was weighed again. The concentration of non-encapsulated surface oil was then adjusted to the original volume of hexane added. The EE was calculated as follows:

$$EE, \% = \frac{A - B}{A} \cdot 100 \quad (1)$$

where A refers to the theoretical amount of encapsulated oil (g) and B to the extractable oil (g).

2.4. Oxidative stability of the spray-dried microcapsules

To investigate the oxidative stability of the microcapsules, 5 g of powder was stored in brown bottles (30 mL and 26-mm inner diameter) at 25 °C in the dark for 6 weeks. Samples were taken every week (week 0, 1, 2, 3, 4, 5, 6) and placed at −80 °C under a nitrogen atmosphere until analysis.

2.4.1. Peroxide value (PV)

First, the fish oil was extracted from the capsules using hexane:2-propanol (1:1, v/v) solvent. For the extraction, 2 g of powder was dissolved in 10 mL of distilled water prior mixing with the extracting solvent and then, the mixture was centrifuged at 670g for 2 min. The extractions were carried out in duplicates. The peroxide value (PV) was quantified on the lipid extracts using the colorimetric ferric-thiocyanate method at 485 nm, according to Drusch et al. (2012), with some modifications. In brief, the extracted oil was diluted in purified 2-propanol prior to the addition of iron–II–chloride and ammonium thiocyanate solutions. Then, the mixture was incubated for 5 min at ambient temperature. Measurements were carried out in duplicates for each lipid extract. Results were expressed in meq O_2 per kg of oil.

2.4.2. Determination of secondary volatile oxidation products (SVOPs)

The content of secondary volatile oxidation products (SVOPs) of the capsules was determined as described by Thomsen et al. (2016), with some modifications. Approximately, 3 g of powder and 30 mg of internal standard (4-methyl-1-pentanol, 30 μg/g water) were added to a 20 mL glass vial and mixed with 7 mL of water. The vials were then placed in the SPME tray and equilibrated for 3 min at 60 °C. The extraction of the volatile compounds was performed in the headspace of the vials by a SPME fiber 50/30 μm CAR/PDMS 57,295-U (Supelco, Bellefonte, USA) during 45 min at 60 °C. The agitation speed was set to 500 rpm. Afterwards, the extracted compounds were transferred from the SPME fiber to the capillary column Zebron™ ZB-1701 column (30 m × ID 0.25 mm × 0.25 μm film thickness, Phenomenex, USA) using helium gas flow (1.0 mL/min) in the split/splitless injector set at 250 °C. The injection was done in the split mode (split 20:1). The GC oven temperature program was as follows: initial temperature of 35 °C for 3 min, increment of 3.0 °C/min to 70 °C, increment of 7.0 °C/min to 200 °C and increment of 15.0 °C/min to 250 °C. This temperature was kept there for 2 min. The released volatile compounds were then identified by MS-library searches in the National Institute of Standards and Technology (NIST) database (NIST v2.0).

2.5. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) by using Statgraphics version 5.1 (Statistical Graphics Corp., Rockville, MD, USA). A multiple sample comparison using Tukey's test was carried out to identify significant differences between means at a level of confidence of 95% ($p < 0.05$).

3. Results and discussion

3.1. Characterization of the spray-dried microcapsules

3.1.1. Oil droplet size distribution (ODSD)

Before drying, the parent emulsions presented a similar oil droplet size distribution (ODSD) with the curves showing a monomodal distribution with the main peak centred at ~0.2 μm and a shoulder, less representative, centred at ~0.7 μm (Fig. 1). Conversely, the reconstituted emulsions showed a bimodal ODSD, although a small shoulder at high diameter values could be also observed (~7 μm). From Fig. 1, it can be clearly observed that for the reconstituted emulsions the proportion of the main peak decreased as the proportion of the shoulder increased, whilst both peaks were displaced to larger diameter values (i.e., the main peak was displaced from ~0.2 μm to ~0.3 μm). An increase in the oil droplet size of reconstituted powders is often related to flocculation/coalescence of the non-encapsulated oil fraction after redispersion, as a result of emulsion destabilization during processing (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007). Thus, our results are indicative that the T20 used as the emulsifier could not efficiently retain the integrity of the oil/water interface for all the oil droplets during spray-drying (Taboada et al., 2021). These results are in agreement with previous studies in which the original fine and monomodal ODSD of a T20-stabilized parent emulsion changed to a bimodal ODSD of larger oil droplets after redispersion of the resulting spray-dried powder in water (Hernández Sánchez, Cuvelier, & Turchiuli, 2016; Rahmani-Manglano, Tirado-Delgado, et al., 2022).

3.1.2. Morphology and size

Fig. 2A–E shows that spherical particles with mostly smooth surfaces were obtained. The mean diameter of the different type of capsules varied from 12.2 ± 7.6 μm to 13.7 ± 7.4 μm ($p > 0.05$), with more than 80% of the particles presenting a size below 20 μm (Fig. 2F). As expected, no significant differences were observed regarding the

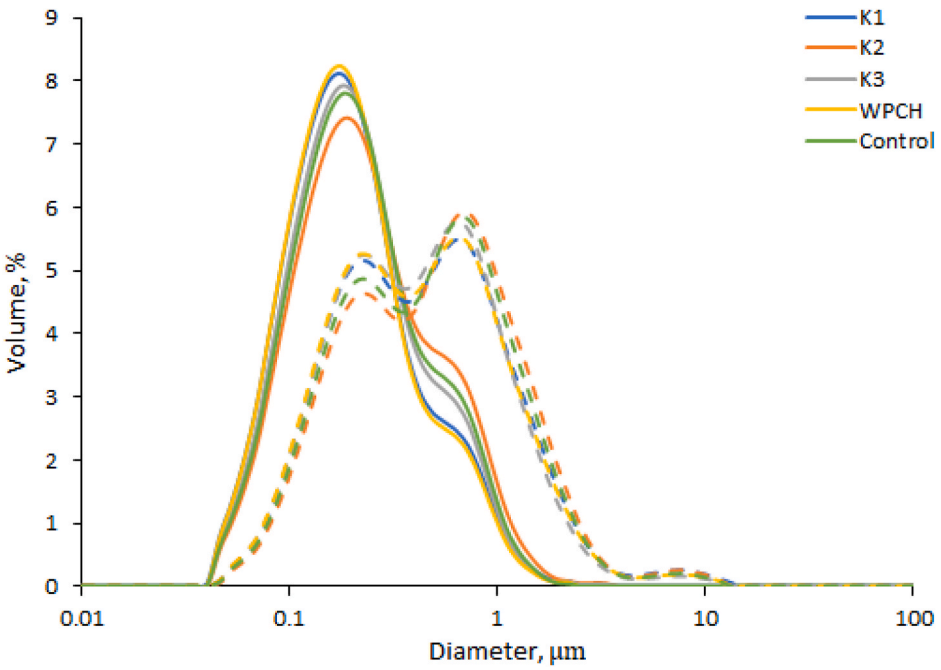

Fig. 1. Oil droplet size distribution (ODSD) of the parent emulsions before drying (solid lines) and the reconstituted emulsions after drying (dashed lines).

morphology and size of the capsules since these properties are influenced by the composition of the emulsions (e.g., type and concentration of the encapsulating agent) and the processing conditions (e.g., inner/outer drying temperature) (Encina et al., 2016). Thus, it was confirmed that at the fixed drying conditions used to produce the capsules, neither the nature (i.e., hydrophobic/hydrophilic) nor the concentration of the antioxidant added to the formulation (i.e., 200 ppm with respect to the oil) affected the microstructure of the particles, as previously reported by other authors (Hernández Sánchez et al., 2016).

3.1.3. Encapsulation efficiency (EE)

High EE values were achieved in all the encapsulated systems studied (EE > 91%, Table 1) indicating a high retention of the oil droplets within the encapsulating matrix during drying. Therefore, taking into account the results of the ODSD of the reconstituted emulsions (Section 3.1.1), it could be speculated that the significantly larger oil droplets found in the reconstituted emulsions ($p < 0.05$) occurred as a result of oil droplets flocculation/coalescence during processing rather than flocculation/coalescence of the non-encapsulated oil fraction due to its low amount (<8% non-encapsulated oil). Therefore, although the integrity of the T20-stabilized oil/water interface was not retained during atomization, the larger fish oil droplets were efficiently trapped within the GS-based encapsulating matrix after drying.

Our results also show that the antioxidant used in the formulation had a minor effect on the EE values despite the statistical analysis results showing significant differences for WPCH and Control samples compared to the CA + CN-based antioxidants (K1, K2, K3) ($p < 0.05$, Table 1). In this line, other authors have reported minor differences in the EE values when producing fish oil-loaded capsules by spray-drying containing antioxidants, the latter not being affected by the amount or type of antioxidant/s used in the formulation (Hogan, O'Riordan, & O'Sullivan, 2003; Serfert et al., 2009). The EE values achieved in the current study are slightly lower than others reported in the literature in which GS was used as the main encapsulating agent (EE > 91%) (Polavarapu, Oliver, Ajlouni, & Augustin, 2011; Rahmani-Manglano et al., 2020; Serfert et al., 2009; Tamm et al., 2015). It should be noted, however, that the aforementioned studies used either modified carbohydrates (e.g., n-octenylsuccinate-derivatised starch) or proteins/protein hydrolysates as emulsifiers (e.g., whey protein concentrate hydrolysate), all exerting film-forming properties contrary to T20.

Taken altogether, from the results on physicochemical properties of the capsules (i.e., ODSD, morphology and size, and EE), we can state that the potential differences on their oxidative stability will arise as a result of the different antioxidant activity of the natural antioxidants added to the formulation (i.e., K1, K2, K3 or WPCH).

3.2. Oxidative stability of the spray-dried microcapsules

3.2.1. Peroxide value (PV)

Fig. 3 shows the content of primary oxidation products (i.e., peroxide value, PV) at the beginning and at the end of the storage time (i.e., 6 weeks of storage at 25 °C). After drying, a significant increase in the PV occurred in all the samples compared to the fresh fish oil (i.e., PV of the capsules in the range 2.8 ± 0.2–5.3 ± 0.5 meq O_2/kg oil after drying). These results further confirm that the onset of lipid oxidation already occurred during processing as a result of the emulsification step and subsequent drying at high temperatures (Baik et al., 2004; Serfert et al., 2009). Nonetheless, different initial oxidation extents could be observed depending on the antioxidant used in the formulation (Fig. 3). The lowest PV after drying was found in K3 sample, followed by K2 and WPCH samples, respectively. On the contrary, the K1 and the Control samples were the most degraded after processing (Fig. 3, $p > 0.05$). As shown in Fig. S1 of the Supplementary Material, the rosemary-based antioxidants were consumed during processing since only ~63–67% of the initial concentration of CA + CN remained after drying. The lowest concentration of CA + CN found corresponded to the K3 sample which, together with the lowest PV found after drying, may be an indicative of a higher consumption of antioxidants during processing. However, K1 and K2 antioxidants were consumed to a similar extent during processing although their initial PV was significantly different (Fig. S1 of the Supplementary Material and Fig. 3, respectively). In liquid heterogeneous systems, such as the wet spray-dried emulsions, the location of the antioxidant highly influences its protective effect against lipid oxidation. In fact, lipophilic and/or amphiphilic antioxidants have been reported to be more efficient than hydrophilic antioxidants since they tend to accumulate near to or at the oil/water interface where lipid oxidation is initiated (Shahidi & Zhong, 2011). In this line, ten Klooster et al. (2022) reported that the PV after spray-drying sunflower oil-in-water

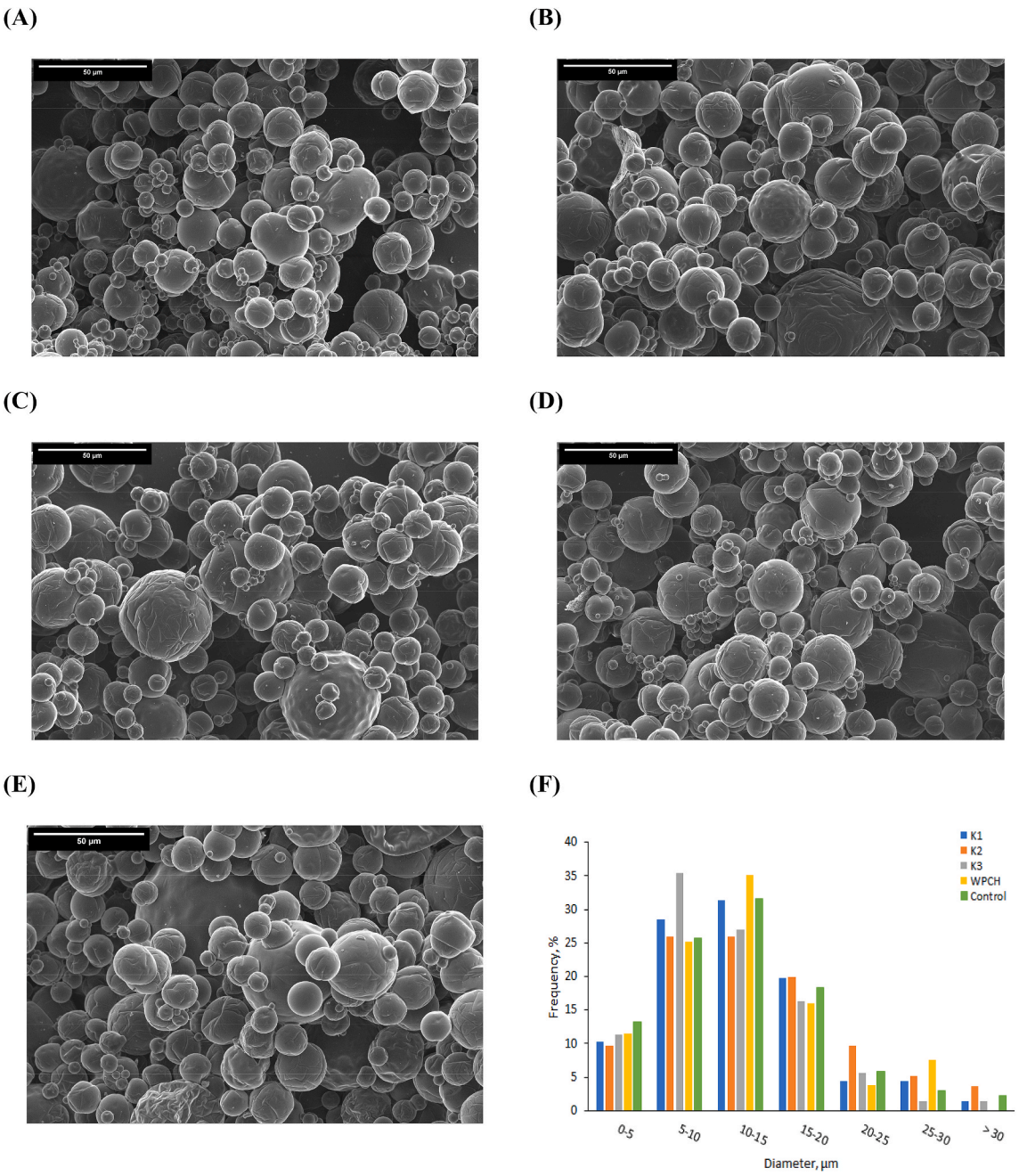

Fig. 2. SEM images **(A–E)** and particle size distribution **(F)** of the spray-dried microcapsules: K1 **(A)**; K2 **(B)**; K3 **(C)**; WPCH **(D)**; Control **(E)**. Scale bar: 50 μm.

Table 1
Encapsulation efficiency of the fish oil-loaded microcapsules. Samples followed by letter, a-b, indicates statistical differences ($p \leq 0.05$) between microcapsules.

Sample	EE, %
K1	92.8 ± 0.1^{a}
K2	92.4 ± 0.3^{a}
K3	92.8 ± 0.3^{a}
WPCH	91.6 ± 0.2^{b}
Control	91.7 ± 0.2^{b}

emulsions stabilized with whey protein isolate in presence of gallic acid decreased as the alkyl chain length of gallates increased (i.e., increasing hydrophobicity of the antioxidant molecule). Furthermore, in a previous study, Serfert et al. (2009) pointed out that lipid oxidation could be significantly reduced during spray-drying processing when a synergistic combination of antioxidants with different mechanisms of action was used in the formulation (e.g., lipophilic antioxidants, synergistic compounds and metal chelator. Therefore, our results are in the same line than those previously reported since the capsules containing the lipid-soluble rosemary extract in combination with tocopherols, ascorbic acid, citric acid and lecithin (K3 sample) prevented the formation of hydroperoxides more efficiently during processing, followed by the lipid-soluble rosemary extract alone (K2 sample) and the whey protein concentrate hydrolysate (WPCH sample), the latter exhibiting surface-active properties (Padial-Domínguez, Espejo-Carpio, García--Moreno, et al., 2020) (Fig. 3). Thus, although the water-dispersible K1 antioxidant was consumed during processing (Fig. S1 of the Supplementary Material), it was less effective in preventing lipid oxidation, presumably due to its tendency towards the aqueous phase of the wet

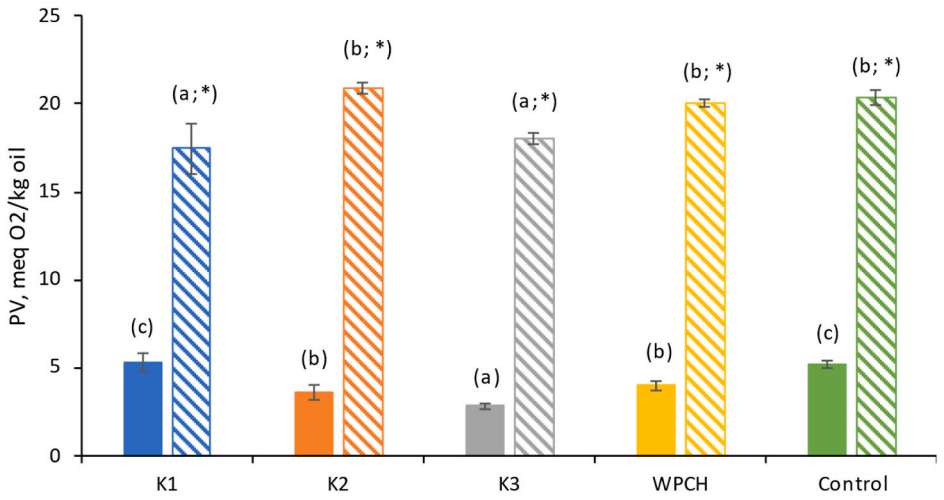

Fig. 3. Peroxide value (PV) of the fish oil-loaded microcapsules stabilized with antioxidants of different polarity during 6 weeks of storage at ambient temperature (25 °C). Samples followed by a letter, a-c, indicates statistical differences ($p \leq 0.05$) between microcapsules at the same storage day. Means within the same sample followed by an asterisk (*) indicates statistical differences ($p \leq 0.05$) between week 0 and week 6.

spray-dried emulsion (Shahidi & Zhong, 2011; ten Klooster et al., 2022).

It is worth mentioning that, although initial lipid oxidation was reduced in the presence of the non-polar/amphiphilic antioxidants (i.e., K2, K3 and WPCH samples), it could not be completely avoided, as also reported by other authors (Baik et al., 2004; Hogan et al., 2003; Serfert et al., 2009; ten Klooster et al., 2022). Moreover, during storage, the presence of antioxidants in the fish oil-loaded capsules (samples K1, K2, K3 and WPCH) did not reduce the formation of lipid peroxides compared to the Control sample since no lag phase was observed for any of the encapsulated systems produced, and the oxidation rate and extent was similar among the samples (i.e., final PV in the range 17.5 ± 1.4–20.9 ± 0.3 meq O_2/kg oil, data not shown). Interestingly, the lowest PV after 6 weeks of storage was found for sample K1. However, low PVs may be indicative of an advanced oxidation state due to hydroperoxides decomposition to secondary oxidation products (e.g., aldehydes) rather than being indicative of a good performance of the antioxidant, as will be discussed below.

3.2.3. *Secondary volatile oxidation products (SVOPs)*

The content of selected secondary volatiles oxidation products (SVOPs) derived from the oxidation of omega-3 PUFAs (e.g., 2-ethylfuran, 1-penten-3-ol, 2-pentenal, 2-hexenal, (*Z*)-4-heptenal, (*E,E*)-2,4-heptadienal and nonanal) was quantified at the beginning (week 0) and at the end (week 6) of the storage time (Fig. 4).

Overall, our results indicate that the water-dispersible rosemary extract (sample K1) was less effective inhibiting lipid oxidation both, during processing and during storage. This was confirmed by the highest PV found after drying for K1 sample (Fig. 3) and by the highest content of SVOPs developed during processing (e.g., 2-pentenal and nonanal) and subsequent storage of the capsules (e.g., 2-ethylfuran, 1-penten-3-ol, 2-pentenal, 2-hexenal, (*Z*)-4-heptenal and nonanal, Fig. 4). This is once again attributed to the partition of K1 antioxidant within the wet (i.e., fish oil-in water emulsion) and subsequently dried (i.e., fish oil-loaded capsules) heterogeneous systems. Due to its polarity, K1 antioxidant is more likely to be dispersed in the aqueous phase of the wet spray-dried emulsion. Therefore, after water removal, K1 antioxidant is expected to be trapped within the glassy encapsulating matrix. In low moisture systems the antioxidant activity is assumed to be diffusion-limited (Barden & Decker, 2013), thus antioxidant molecules located within the encapsulating matrix – where the mobility is significantly limited – might be less effective preventing lipid oxidation. Indeed, previous studies which evaluated the effect of polar antioxidants and their non-polar analogues (e.g., α-tocopherol and Trolox or gallic acid and its ester derivatives) in dried encapsulated systems, reported that non-polar antioxidants, readily present in the oil phase, prevented lipid oxidation during storage more efficiently than their polar counterpart (Hogan et al., 2003; Velasco, Holgado, Dobarganes, & Márquez-Ruiz, 2009). The latter is in agreement with our observations since the oxidative stability of the fish oil-loaded capsules was significantly enhanced in the presence of the lipid-soluble rosemary extract analogue (K2 sample) compared to its polar equivalent (K1 sample) as confirmed by the significantly lower content of all the selected SVOPs found after storage (Fig. 4). Interestingly, although additional stabilization with mixed antioxidants (K3 sample) prevented lipid oxidation more efficiently during processing (i.e., lowest initial PV, Fig. 3), it did not significantly enhance the oxidative stability of the capsules during storage compared to single addition of the lipid-soluble rosemary extract (K2 sample) (Fig. 4). This phenomenon was already observed by other authors who found that the synergistic effects derived from the combination of antioxidants which efficiently stabilized bulk or emulsified oils did not notably improve the oxidative stability of the encapsulated oil due to the different nature of the oil-loaded heterogeneous systems (i.e., wet heterogeneous system or dehydrated heterogeneous system) (Serfert et al., 2009; Velasco, Dobarganes, & Márquez-Ruiz, 2000). The lower protective effect of the combined antioxidants was attributed to the lower contribution of the hydrosoluble constituents, hindered by their reduced mobility in the dehydrated system compared to the wet system (Serfert et al., 2009; Velasco et al., 2000). Therefore, our results suggest that despite the lipid-soluble nature of K3 antioxidant, the components of the antioxidants mixture partitioned within the capsules based on their polarity (e.g., ascorbic acid and citric acid are polar antioxidants whilst tocopherols are non-polar), resulting in a lower overall protective effect of the encapsulated fish oil.

As for WPCH, which exhibits both antioxidant and emulsifying properties, it was able to reduce lipid oxidation of the encapsulated fish oil as confirmed by the lower amount of the selected SVOPs (e.g., 2-pentenal, nonanal, 1-penten-3-ol) developed after storage compared to the other antioxidant-containing samples (Fig. 4). These results contrast with previous studies which reported that amphiphilic antioxidants were less efficient preventing lipid oxidation in oil-loaded encapsulated systems than more non-polar antioxidants (e.g., α-tocopherol vs. ascorbyl palmitate or propyl gallate vs. octyl gallate) (Baik et al., 2004; ten Klooster et al., 2022). For instance, ten Klooster et al. (2022) argued that the preferred partitioning of propyl gallate at the oil/water interface of the spray-dried emulsion was detrimental in terms of its antioxidant activity in the encapsulated system. This was attributed either

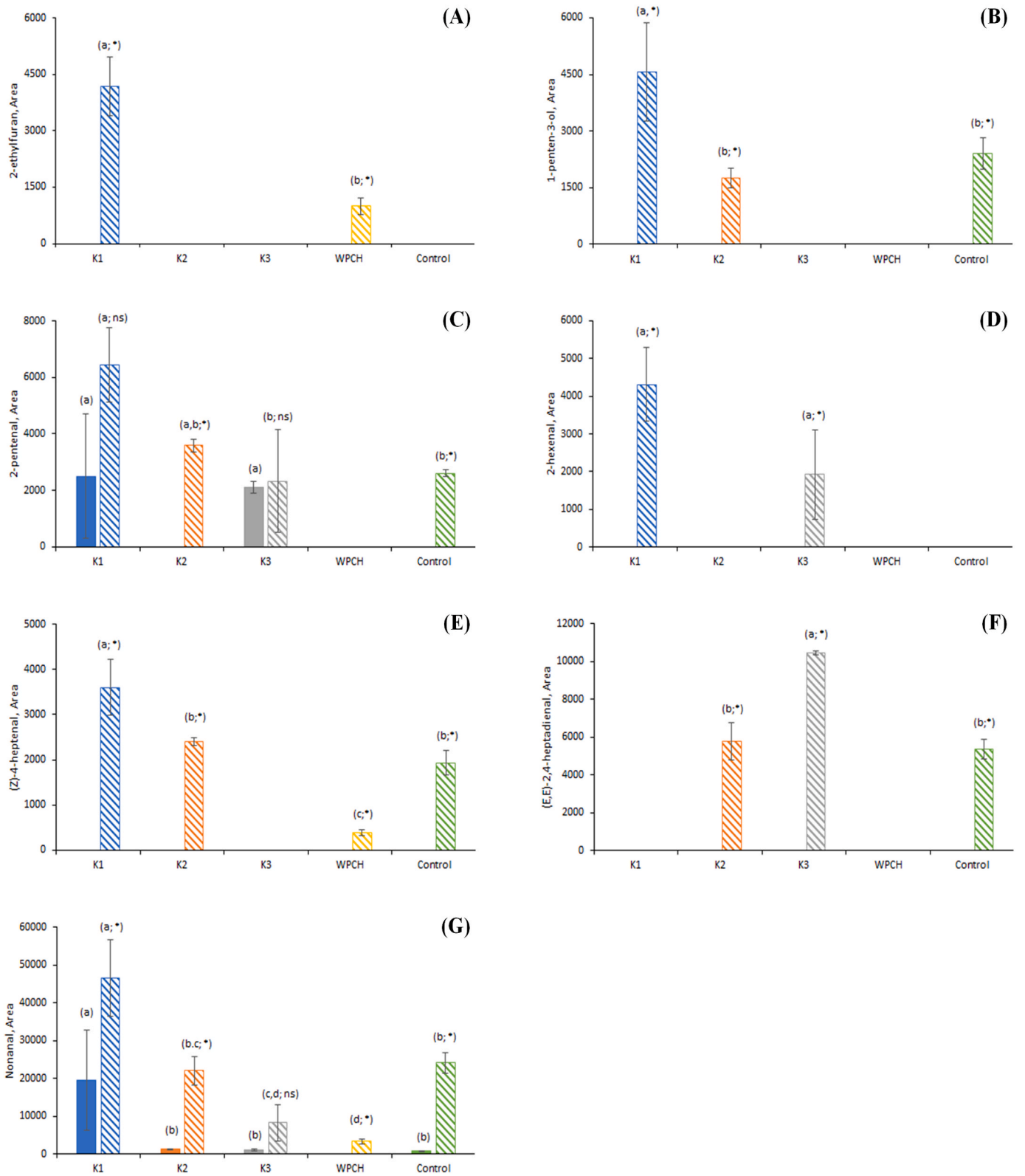

Fig. 4. Secondary volatile oxidation products (SVOPs) content of the fish oil-loaded microcapsules after production (filled bars) and after 6 weeks of storage (dashed bars) at ambient temperature (25 °C). Samples followed by a letter, a-d, indicates statistical differences ($p \leq 0.05$) between microcapsules. Means within the same sample followed by an asterisk (*) indicates statistical differences ($p \leq 0.05$) between week 0 and week 6. Means within the same sample followed by "ns" indicates no statistical differences ($p > 0.05$) between week 0 and week 6.

to: i) a possible glassy state of the interface after drying or to ii) preferential location of the antioxidant within the glassy encapsulating matrix upon drying, both reducing the mobility of the antioxidant towards oxidizing lipids. However, the latter was not observed in the current study. It is well known that the distribution of the antioxidant within wet heterogeneous systems strongly depends on the concentration and type of the emulsifier used in the formulation (Shahidi & Zhong, 2011; Stöckmann, Schwarz, & Huynh-Ba, 2000). Thus, antioxidant molecules with amphiphilic properties may not locate at the oil/-water interface depending on the nature of the emulsifier used and how much interfacial area is covered. WPCH has been demonstrated to exhibit both high interfacial adsorption and strong interactions WPCH-oil due to its enhanced surface hydrophobicity as a consequence of the higher exposure of non-polar amino acids (Rahmani-Manglano, Jones, et al., 2022; Ruiz-Álvarez et al., 2022). Therefore, co-adsorption of WPCH at the oil/water interface of the wet emulsion stabilized with low concentration of T20 (i.e., 0.35 wt% of T20) cannot be ruled out and, in consequence, its location at the oil/matrix interface of the subsequently dehydrated system. Furthermore, based on its amino acid profile, WPCH has been proved to exhibit high antioxidant activity showing combined mechanisms of action as metal chelator and radical scavenger in its polar part (i.e., by action of His and Tyr) and reducing power and radical scavenger in its non-polar part (i.e., by action of Met) (Elias, Kellerby, & Decker, 2008; Padial-Domínguez, Espejo-Carpio, García-Moreno, et al., 2020; Rahmani-Manglano et al., 2020). Thus, contrary to propyl gallate which shows radical scavenging activity only by its polar head (i.e., phenolic group) (Garrido et al., 2012), WPCH is capable to: i) scavenge free radicals in the oil phase of the dried system and ii) to inhibit lipid oxidation through different antioxidant paths when located at the oil/matrix interface. It should be borne in mind that the commercial fish oil used in the current study already had a high concentration of non-polar antioxidants (i.e., tocopherols) contrary to the aforementioned study, which used striped sunflower oil (ten Klooster et al., 2022). Thus, the antioxidant activity of WPCH used as amphiphilic antioxidant in this work was combined with the antioxidant activity of the tocopherols already present in the fish oil. This could further explain the differences observed between the two studies together with the enhanced oxidative stability observed for the WPCH-containing samples in the current study.

4. Conclusions

The influence of the polarity of natural antioxidants on the oxidative stability of spray-dried fish oil-loaded capsules was investigated. All the capsules obtained had similar morphology, size and encapsulation efficiency (EE > 91%), meaning that the physicochemical properties of the encapsulated systems were not influenced by the antioxidant used in the formulation. However, the polar rosemary extract studied was less effective preventing lipid oxidation during processing and later storage of the capsules compared to the non-polar rosemary extracts and WPCH (i.e., an amphiphilic antioxidant). This was attributed to the preferred partitioning of the polar rosemary extract within the aqueous phase of the wet emulsion, far from where lipid oxidation is initiated, and subsequently within the glassy matrix of the dried capsules, where the mobility of the antioxidant is significantly reduced. Interestingly, additional chemical stabilization in the presence of mixed antioxidants (i.e., rosemary extract, mixed tocopherols, ascorbic acid, citric acid and lecithin) with different mechanisms of action did not significantly reduce lipid oxidation during storage compared to the capsules containing the lipid-soluble rosemary extract alone. The latter was discussed on the basis of the partition of the different mixed antioxidants within the dried capsules based on their polarity (e.g., polar components trapped within the glassy matrix) resulting in a reduced combinatory effect. Nonetheless, WPCH exhibiting high surface-active properties and combined antioxidant mechanisms of action in their polar (i.e., metal chelating and radical scavenging activity) and non-polar part (e.g., radical scavenging activity and reducing power), significantly enhanced the oxidative stability of the encapsulated commercial fish oil containing tocopherols.

Author statement

Nor E. Rahmani-Manglano: Methodology, Investigation, Formal analysis, Writing - original draft. **Pedro J. García-Moreno**: Conceptualization, Methodology, Supervision, Writing - review and editing. **Raúl Pérez-Gálvez**: Conceptualization, Methodology, Supervision, Writing - review and editing. **Emilia M. Guadix**: Conceptualization, Supervision, Writing - review and editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was funded by the I + D + i project CTQ2017-87076-R from the Spanish Ministry of Science and Innovation MCIN/AEI/10.13039/501100011033/. N. E. Rahmani-Manglano acknowledges a FPI grant PRE2018-084861 funded by MCIN/AEI/10.13039/501100011033. The authors are very grateful to Wu Chenxing for her help on the production and characterization of the encapsulated systems. The authors greatly acknowledge Kalsec, Inc. for donating the commercial antioxidants used in the study and for determining the content of CA + CN in the capsules after spray-drying. Funding for open access charge: Universidad de Granada/CBUA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2023.103074.

References

Anwar, F., & Qadir, R. (2021). Carnosic acid and carnosol. In *A centum of valuable plant bioactives* (pp. 261–274). Elsevier. https://doi.org/10.1016/B978-0-12-822923-1.00012-1.

Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science and Technology, 25*(1), 24–33. https://doi.org/10.1016/j.tifs.2011.12.002

Aslam, A., & Schroën, K. (2023). Lipid oxidation in food emulsions: A review dedicated to the role of the interfacial area. *Current Opinion in Food Science, 51*, Article 101009. https://doi.org/10.1016/j.cofs.2023.101009

Baik, M. Y., Suhendro, E. L., Nawar, W. W., McClements, D. J., Decker, E. A., & Chinachoti, P. (2004). Effects of antioxidants and humidity on the oxidative stability of microencapsulated fish oil. *JAOCS, Journal of the American Oil Chemists' Society, 81*(4), 355–360. https://doi.org/10.1007/s11746-004-0906-7

Barden, L., & Decker, E. A. (2013). Lipid oxidation in low-moisture food: A review. In *Critical reviews in food science and nutrition* (Vol. 56, pp. 2467–2482). Taylor and Francis Inc. https://doi.org/10.1080/10408398.2013.848833, 15.

Berton-Carabin, C. C., Ropers, M. H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive Reviews in Food Science and Food Safety, 13*(5), 945–977. https://doi.org/10.1111/1541-4337.12097

Drusch, S., Serfert, Y., Berger, A., Shaikh, M. Q., Rätzke, K., Zaporojtchenko, V., et al. (2012). New insights into the microencapsulation properties of sodium caseinate and hydrolyzed casein. *Food Hydrocolloids, 27*(2), 332–338. https://doi.org/10.1016/j.foodhyd.2011.10.001

Drusch, S., Serfert, Y., Scampicchio, M., Schmidt-Hansberg, B., & Schwarz, K. (2007). Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. *Journal of Agricultural and Food Chemistry, 55*(26), 11044–11051. https://doi.org/10.1021/jf072536a

Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical Reviews in Food Science and Nutrition, 48*(5), 430–441. https://doi.org/10.1080/10408390701425615

Encina, C., Vergara, C., Giménez, B., Oyarzún-Ampuero, F., & Robert, P. (2016). Conventional spray-drying and future trends for the microencapsulation of fish oil. *Trends in Food Science and Technology, 56*, 46–60. https://doi.org/10.1016/j.tifs.2016.07.014

García-Moreno, P. J., Damberg, C., Chronakis, I. S., & Jacobsen, C. (2017). Oxidative stability of pullulan electrospun fibers containing fish oil: Effect of oil content and natural antioxidants addition. *European Journal of Lipid Science and Technology, 119* (12). https://doi.org/10.1002/ejlt.201600305

Garrido, J., Garrido, E. M., & Borges, F. (2012). Studies on the food additive propyl gallate: Synthesis, structural characterization, and evaluation of the antioxidant activity. *Journal of Chemical Education, 89*(1), 130–133. https://doi.org/10.1021/ed900025s

Hernández Sánchez, M. del R., Cuvelier, M. E., & Turchiuli, C. (2016). Effect of α-tocopherol on oxidative stability of oil during spray drying and storage of dried emulsions. *Food Research International, 88*, 32–41. https://doi.org/10.1016/j.foodres.2016.04.035

Hogan, S. A., O'Riordan, E. D., & O'Sullivan, M. (2003). Microencapsulation and oxidative stability of spray-dried fish oil emulsions. *Journal of Microencapsulation, 20* (5), 675–688. https://doi.org/10.1080/0265204031000151974

Hopia, A. I., Huang, S.-W., Schwarz, K., German, J. B., & Frankel, E. N. (1996). *Effect of different lipid systems on antioxidant activity of rosemary constituents carnosol and carnosic acid with and without r-tocopherol*. https://doi.org/10.1021/jf950777p

Hrebień-Filisińska, A. (2021). Application of natural antioxidants in the oxidative stabilization of fish oils: A mini-review. *Journal of Food Processing and Preservation, 45*(4), 1–12. https://doi.org/10.1111/jfpp.15342

Johnson, D. R., & Decker, E. A. (2015). The role of oxygen in lipid oxidation reactions: A review. *Annual Review of Food Science and Technology, 6*, 171–190. https://doi.org/10.1146/annurev-food-022814-015532

ten Klooster, S., Villeneuve, P., Bourlieu-Lacanal, C., Durand, E., Schroën, K., & Berton-Carabin, C. (2022). Alkyl chain length modulates antioxidant activity of gallic acid esters in spray-dried emulsions. *Food Chemistry, 387*. https://doi.org/10.1016/j.foodchem.2022.132880

Laguerre, M., Bayrasy, C., Panya, A., Weiss, J., McClements, D. J., Lecomte, J., et al. (2015). What makes good antioxidants in lipid-based systems? The next theories beyond the polar paradox. *Critical Reviews in Food Science and Nutrition, 55*(2), 183–201. https://doi.org/10.1080/10408398.2011.650335

Padial-Domínguez, M., Espejo-Carpio, F. J., García-Moreno, P. J., Jacobsen, C., & Guadix, E. M. (2020). Protein derived emulsifiers with antioxidant activity for stabilization of omega-3 emulsions. *Food Chemistry, 329*, Article 127148. https://doi.org/10.1016/j.foodchem.2020.127148

Padial-Domínguez, M., Espejo-Carpio, F. J., Pérez-Gálvez, R., Guadix, A., & Guadix, E. M. (2020). Optimization of the emulsifying properties of food protein hydrolysates for the production of fish oil-in-water emulsions. *Foods, 9*(5). https://doi.org/10.3390/foods9050636

Patel, A., Desai, S. S., Mane, V. K., Enman, J., Rova, U., Christakopoulos, P., et al. (2022). Futuristic food fortification with a balanced ratio of dietary ω-3/ω-6 omega fatty acids for the prevention of lifestyle diseases. In *Trends in food science and Technology* (Vol. 120, pp. 140–153). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2022.01.006.

Polavarapu, S., Oliver, C. M., Ajlouni, S., & Augustin, M. A. (2011). Physicochemical characterisation and oxidative stability of fish oil and fish oil-extra virgin olive oil microencapsulated by sugar beet pectin. *Food Chemistry, 127*(4), 1694–1705. https://doi.org/10.1016/j.foodchem.2011.02.044

Rahmani-Manglano, N. E., González-Sánchez, I., García-Moreno, P. J., Espejo-Carpio, F. J., Jacobsen, C., & Guadix, E. M. (2020). Development of fish oil-loaded microcapsules containing whey protein hydrolysate as film-forming material for fortification of low-fat mayonnaise. *Foods, 9*(5), 1–21. https://doi.org/10.3390/foods9050545

Rahmani-Manglano, N. E., Jones, N. C., Hoffmann, S. V., Guadix, E. M., Pérez-Gálvez, R., Guadix, A., et al. (2022). Structure of whey protein hydrolysate used as emulsifier in wet and dried oil delivery systems: Effect of pH and drying processing. *Food Chemistry, 390*, Article 133169. https://doi.org/10.1016/j.foodchem.2022.133169

Rahmani-Manglano, N. E., Tirado-Delgado, M., García-Moreno, P. J., Guadix, A., & Guadix, E. M. (2022). Influence of emulsifier type and encapsulating agent on the in vitro digestion of fish oil-loaded microcapsules produced by spray-drying. *Food Chemistry, 392*, Article 133257. https://doi.org/10.1016/j.foodchem.2022.133257

Ruiz-Álvarez, J. M., del Castillo-Santaella, T., Maldonado-Valderrama, J., Guadix, A., Guadix, E. M., & García-Moreno, P. J. (2022). pH influences the interfacial properties of blue whiting (M. poutassou) and whey protein hydrolysates determining the physical stability of fish oil-in-water emulsions. *Food Hydrocolloids, 122*, Article 107075. https://doi.org/10.1016/j.foodhyd.2021.107075

Sandhya, K., Leena, M. M., Moses, J. A., & Anandharamakrishnan, C. (2023). Edible oil to powder technologies: Concepts and advances. *Food Bioscience, 53*, Article 102567. https://doi.org/10.1016/j.fbio.2023.102567

Santos, D., Maurício, A. C., Sencadas, V., Santos, J. D., Fernandes, M. H., & Gomes, P. S. (2018). *Spray drying: An overview*. Biomaterials - Physics and Chemistry - New Edition. https://doi.org/10.5772/intechopen.72247

Serfert, Y., Drusch, S., & Schwarz, K. (2009). Chemical stabilisation of oils rich in long-chain polyunsaturated fatty acids during homogenisation, microencapsulation and storage. *Food Chemistry, 113*(4), 1106–1112. https://doi.org/10.1016/j.foodchem.2008.08.079

Shahidi, F., & Zhong, Y. (2011). Revisiting the polar paradox theory: A critical overview. *Journal of Agricultural and Food Chemistry, 59*(8), 3499–3504. https://doi.org/10.1021/jf104750m

Sørensen, A.-D. M., García-Moreno, P. J., Yesiltas, B., & Jacobsen, C. (2021). Introduction to delivery systems and stability issues. In *Omega-3 delivery systems* (pp. 107–117). Elsevier. https://doi.org/10.1016/b978-0-12-821391-9.00015-6.

Stöckmann, H., Schwarz, K., & Huynh-Ba, T. (2000). The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *AOCS Press, 77*(5), 535–542. https://doi.org/10.1007/s11746-000-0085-6

Taboada, M. L., Heiden-Hecht, T., Brückner-Gühmann, M., Karbstein, H. P., Drusch, S., & Gaukel, V. (2021). Spray drying of emulsions: Influence of the emulsifier system on changes in oil droplet size during the drying step. *Journal of Food Processing and Preservation, 45*(9), 1–11. https://doi.org/10.1111/jfpp.15753

Tamm, F., Gies, K., Diekmann, S., Serfert, Y., Strunskus, T., Brodkorb, A., et al. (2015). Whey protein hydrolysates reduce autoxidation in microencapsulated long chain polyunsaturated fatty acids. *European Journal of Lipid Science and Technology, 117* (12), 1960–1970. https://doi.org/10.1002/ejlt.201400574

Thomsen, B. R., Yesiltas, B., Sørensen, A. D. M., Hermund, D. B., Glastrup, J., & Jacobsen, C. (2016). Comparison of three methods for extraction of volatile lipid oxidation products from food matrices for GC–MS analysis. *JAOCS, Journal of the American Oil Chemists' Society, 93*(7), 929–942. https://doi.org/10.1007/s11746-016-2837-2

Velasco, J., Dobarganes, M. C., & Márquez-Ruiz, G. (2000). Application of the accelerated test Rancimat to evaluate oxidative stability of dried microencapsulated oils. *Grasas Y Aceites, 51*(4), 261–267. https://doi.org/10.3989/gya.2000.v51.i4.422

Velasco, J., Dobarganes, C., & Márquez-Ruiz, G. (2003). Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas Y Aceites, 54*(3), 304–314.

Velasco, J., Holgado, F., Dobarganes, C., & Márquez-Ruiz, G. (2009). Antioxidant activity of added phenolic compounds in freeze-dried microencapsulated sunflower oil. *JAOCS, Journal of the American Oil Chemists' Society, 86*(5), 445–452. https://doi.org/10.1007/s11746-009-1365-8